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A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al.* *Trends in Biotechnol.* 11, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem.* 263, 621-24 (1988);

Wu *et al.*, *J. Biol. Chem.* 269, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem.* 266, 338-42 (1991).

Determination of a Therapeutically Effective Dose

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The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases serine-threonine protein kinase activity relative to the serine-threonine protein kinase activity which occurs in the absence of the therapeutically effective dose.

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For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

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Therapeutic efficacy and toxicity, *e.g.*, ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀.

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Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

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The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg,

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about 1 μ g to about 2 mg, about 5 μ g to about 500 μ g, and about 20 μ g to about 100 μ g of DNA.

5 If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

10 Preferably, a reagent reduces expression of a serine-threonine protein kinase gene or the activity of a serine-threonine protein kinase polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a serine-threonine protein kinase gene or the activity of a serine-threonine protein kinase polypeptide can be assessed using methods well known in
15 the art, such as hybridization of nucleotide probes to serine-threonine protein kinase-specific mRNA, quantitative RT-PCR, immunologic detection of a serine-threonine protein kinase polypeptide, or measurement of serine-threonine protein kinase activity.

20 In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the
25 treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in
30 need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

Human serine-threonine protein kinase also can be used in diagnostic assays for
5 detecting diseases and abnormalities or susceptibility to diseases and abnormalities
related to the presence of mutations in the nucleic acid sequences which encode the
enzyme. For example, differences can be determined between the cDNA or geno-
mic sequence encoding serine-threonine protein kinase in individuals afflicted with a
disease and in normal individuals. If a mutation is observed in some or all of the
10 afflicted individuals but not in normal individuals, then the mutation is likely to be
the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be
revealed by the direct DNA sequencing method. In addition, cloned DNA segments
15 can be employed as probes to detect specific DNA segments. The sensitivity of this
method is greatly enhanced when combined with PCR. For example, a sequencing
primer can be used with a double-stranded PCR product or a single-stranded template
molecule generated by a modified PCR. The sequence determination is performed
by conventional procedures using radiolabeled nucleotides or by automatic
20 sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection
of alteration in electrophoretic mobility of DNA fragments in gels with or without
denaturing agents. Small sequence deletions and insertions can be visualized, for
25 example, by high resolution gel electrophoresis. DNA fragments of different
sequences can be distinguished on denaturing formamide gradient gels in which the
mobilities of different DNA fragments are retarded in the gel at different positions
according to their specific melting or partial melting temperatures (*see, e.g., Myers et
al., Science 230, 1242, 1985*). Sequence changes at specific locations can also be
30 revealed by nuclease protection assays, such as RNase and S 1 protection or the
chemical cleavage method (*e.g., Cotton et al., Proc. Natl. Acad. Sci. USA 85, 4397-*

4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing,
5 mutations can also be detected by *in situ* analysis.

Altered levels of a serine-threonine protein kinase also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill
10 in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated
15 herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1*Detection of serine-threonine protein kinase activity*

5 For high level expression of a FLAG-tagged serine-threonine protein kinase polypeptide, COS-1 cells are transfected with the expression vector serine-threonine protein kinase polypeptide (expressing the DNA-sequence of ID NO: 1) using the calcium phosphate method. After 5h, the cells are infected with recombinant vaccinia virus vTF7-3 (10 plaque-forming units/cell). The cells are harvested 20h after
10 infection and lysed in 50 mM Tris, pH 7,5, 5 mM MgCl₂, 0,1% Nonidet P-40, 0,5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin. Serine-threonine protein kinase polypeptide is immunoprecipitated from the lysate using anti-FLAG antibodies. In vitro kinase assay and phosphoamino acid analysis are performed in a volume of 40 µl with immunoprecipitated FLAG-serine-threonine
15 protein kinase polypeptide in 50 mM Tris-HCl, pH 8,0, 50 mM NaCl, 5 mM MgCl₂, 1 mM dithiothreitol. The reaction is started by the addition of 4 µl of 1 mM ATP supplemented with 5 µCi of (-32P)ATP and incubated for 30 min at 37°C. Afterward, the samples are subjected to SDS-PAGE and phosphorylated proteins are detected by autoradiography. Histone type III-S, casein, bovine serum albumin, or
20 myelin basic proteins are used as substrates. It is shown that the polypeptide with the amino acid sequence of SEQ ID NO.: 2 has serine-threonine protein kinase activity.

EXAMPLE 2*Expression of recombinant human serine-threonine protein kinase*

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The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human serine-threonine protein kinase polypeptides in yeast. The serine-threonine protein kinase-encoding DNA sequence is derived from SEQ ID NO:1. Before insertion into vector pPICZB, the DNA
30 sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter

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tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in
5 *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography
10 (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human serine-threonine protein kinase polypeptide is obtained.

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EXAMPLE 3

Identification of test compounds that bind to serine-threonine protein kinase polypeptides

20 Purified serine-threonine protein kinase polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human serine-threonine protein kinase polypeptides comprise the amino acid sequence shown in SEQ ID NO:2. The test
25 compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a serine-threonine protein kinase polypeptide is
30 detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to

fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a serine-threonine protein kinase polypeptide.

EXAMPLE 4

5 *Identification of a test compound which decreases serine-threonine protein kinase gene expression*

A test compound is administered to a culture of human cells transfected with a serine- threonine protein kinase expression construct and incubated at 37 °C for 10 to
10 45 minutes. A culture of the same type of cells which have not been transfected is incubated for the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*,
5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and
15 hybridized with a ³²P-labeled serine-threonine protein kinase-specific probe at 65 °C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1. A test compound which decreases the serine-threonine protein kinase-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of serine-
20 threonine protein kinase gene expression.

EXAMPLE 5

Identification of a test compound which decreases human serine-threonine protein kinase activity

25 Cellular extracts from the human colon cancer cell line HCT116 are contacted with test compounds from a small molecule library and assayed for human serine-threonine protein kinase activity. Control extracts, in the absence of a test compound, also are assayed. Kinase activity can be measured, for example, as taught in
30 Trost *et al.*, *J. Biol. Chem.* 275, 7373-77, 2000; Hayashi *et al.*, *Biochem. Biophys. Res. Commun.* 264, 449-56, 1999; Masure *et al.*, *Eur. J. Biochem.* 265, 353-60, 1999;

and Mukhopadhyay *et al.*, *J. Bacteriol.* 181, 6615-22, 1999. A test compound which decreases serine-threonine protein kinase activity of the extract relative to the control extract by at least 20% is identified as a serine-threonine protein kinase inhibitor.

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EXAMPLE 6

Tissue-specific expression of serine/threonine protein kinase

10 The qualitative expression pattern of serine/threonine protein kinase in various tissues is determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

15 To demonstrate that serine/threonine protein kinase is involved in cancer, expression is determined in the following tissues: adrenal gland, bone marrow, brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, and peripheral blood lymphocytes. Expression in the following cancer cell lines also is determined: DU-145 (prostate), NCI-H125 (lung), HT-29 (colon), COLO-205 (colon), A-549 (lung),
20 NCI-H460 (lung), HT-116 (colon), DLD-1 (colon), MDA-MD-231 (breast), LS174T (colon), ZF-75 (breast), MDA-MN-435 (breast), HT-1080, MCF-7 (breast), and U87. Matched pairs of malignant and normal tissue from the same patient also are tested.

25 To demonstrate that serine/threonine protein kinase is involved in the disease process of COPD, the initial expression panel consists of RNA samples from respiratory tissues and inflammatory cells relevant to COPD: lung (adult and fetal), trachea, freshly isolated alveolar type II cells, cultured human bronchial epithelial cells, cultured small airway epithelial cells, cultured bronchial smooth muscle cells, cultured H441 cells (Clara-like), freshly isolated neutrophils and monocytes, and cultured
30 monocytes (macrophage-like). Body map profiling also is carried out, using total RNA panels purchased from Clontech. The tissues are adrenal gland, bone marrow,

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brain, colon, heart, kidney, liver, lung, mammary gland, pancreas, prostate, salivary gland, skeletal muscle, small intestine, spleen, stomach, testis, thymus, trachea, thyroid, and uterus.

5 To demonstrate that serine/threonine protein kinase is involved in the disease process of obesity, expression is determined in the following tissues: subcutaneous adipose tissue, mesenteric adipose tissue, adrenal gland, bone marrow, brain (cerebellum, spinal cord, cerebral cortex, caudate, medulla, substantia nigra, and putamen), colon, fetal brain, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, 10 salivary gland, skeletal muscle small intestine, spleen, stomach, testes, thymus, thyroid trachea, and uterus. Neuroblastoma cell lines SK-Nr-Be (2), Hr, Sk-N-As, HTB-10, IMR-32, SNSY-5Y, T3, SK-N-D2, D283, DAOY, CHP-2, U87MG, BE(2)C, T986, KANTS, MO59K, CHP234, C6 (rat), SK-N-F1, SK-PU-DW, PFSK-1, BE(2)M17, and MCIXC also are tested for serine/threonine protein kinase 15 expression. As a final step, the expression of serine/threonine protein kinase in cells derived from normal individuals with the expression of cells derived from obese individuals is compared.

To demonstrate that serine/threonine protein kinase is involved in the disease process 20 of diabetes, the following whole body panel is screened to show predominant or relatively high expression: subcutaneous and mesenteric adipose tissue, adrenal gland, bone marrow, brain, colon, fetal brain, heart, hypothalamus, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, and uterus. Human 25 islet cells and an islet cell library also are tested. As a final step, the expression of serine/threonine protein kinase in cells derived from normal individuals with the expression of cells derived from diabetic individuals is compared.

To demonstrate that serine/threonine protein kinase is involved in CNS disorders, the 30 following tissues are screened: fetal and adult brain, muscle, heart, lung, kidney, liver, thymus, testis, colon, placenta, trachea, pancreas, kidney, gastric mucosa,

colon, liver, cerebellum, skin, cortex (Alzheimer's and normal), hypothalamus, cortex, amygdala, cerebellum, hippocampus, choroid, plexus, thalamus, and spinal cord.

5 *Quantitative expression profiling.* Quantitative expression profiling is performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology* 10, 413-17, 1992, and Higuchi *et al.*, *BioTechnology* 11, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template
10 copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and
15 a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 7276-80, 1991). Because the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res.* 6, 986-94, 1996, and Gibson *et al.*, *Genome*
20 *Res.* 6, 995-1001, 1996).

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission
25 spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700.

30 *RNA extraction and cDNA preparation.* Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" were extracted

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from autaptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

5 Fifty µg of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/µl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/µl RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10mM MgCl₂; 50 mM NaCl; and 1 mM DTT.

10 After incubation, RNA is extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of 3 M NaAcetate, pH5.2, and 2 volumes of ethanol.

15 Fifty µg of each RNA from the autaptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectrophotometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the manufacturer's protocol. The final concentration of RNA in the reaction mix is 200ng/µL. Reverse transcription is carried out with 2.5µM of random hexamer primers.

20 *TaqMan quantitative analysis.* Specific primers and probe are designed according to the recommendations of PE Applied Biosystems; the probe can be labeled at the 5' end FAM (6-carboxy-fluorescein) and at the 3' end with TAMRA (6-carboxy-tetramethyl-rhodamine). Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

25 Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

30 The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from

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20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng cDNA; and water to 25 µl.

Each of the following steps are carried out once: pre PCR, 2 minutes at 50° C, and
5 10 minutes at 95°C. The following steps are carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are
10 processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

EXAMPLE 7

In vivo testing of compounds/target validation

1. Pain:

Acute Pain

20 Acute pain is measured on a hot plate mainly in rats. Two variants of hot plate testing are used: In the classical variant animals are put on a hot surface (52 to 56 °C) and the latency time is measured until the animals show nocifensive behavior, such as stepping or foot licking. The other variant is an increasing temperature hot plate where the experimental animals are put on a surface of neutral temperature.
25 Subsequently this surface is slowly but constantly heated until the animals begin to lick a hind paw. The temperature which is reached when hind paw licking begins is a measure for pain threshold.

Compounds are tested against a vehicle treated control group. Substance application
30 is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Persistent Pain

Persistent pain is measured with the formalin or capsaicin test, mainly in rats. A solution of 1 to 5% formalin or 10 to 100 µg capsaicin is injected into one hind paw of the experimental animal. After formalin or capsaicin application the animals show nocifensive reactions like flinching, licking and biting of the affected paw. The number of nocifensive reactions within a time frame of up to 90 minutes is a measure for intensity of pain.

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to formalin or capsaicin administration.

Neuropathic Pain

Neuropathic pain is induced by different variants of unilateral sciatic nerve injury mainly in rats. The operation is performed under anesthesia. The first variant of sciatic nerve injury is produced by placing loosely constrictive ligatures around the common sciatic nerve. The second variant is the tight ligation of about the half of the diameter of the common sciatic nerve. In the next variant, a group of models is used in which tight ligations or transections are made of either the L5 and L6 spinal nerves, or the L₅ spinal nerve only. The fourth variant involves an axotomy of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact whereas the last variant comprises the axotomy of only the tibial branch leaving the sural and common nerves uninjured. Control animals are treated with a sham operation.

Postoperatively, the nerve injured animals develop a chronic mechanical allodynia, cold allodynia, as well as a thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC

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Inc.-Life Science Instruments, Woodland Hills, SA, USA; Electronic von Frey System, Somedic Sales AB, Hörby, Sweden). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy), or by means of a cold plate of 5 to 10 °C where the nocifensive reactions of the affected hind paw are counted as a measure of pain intensity. A further test for cold induced pain is the counting of nocifensive reactions, or duration of nocifensive responses after plantar administration of acetone to the affected hind limb. Chronic pain in general is assessed by registering the circadian rhythms in activity (Surjo and Arndt, Universität zu Köln, Cologne, Germany), and by scoring differences in gait (foot print patterns; FOOTPRINTS program, Klapdor et al., 1997. A low cost method to analyze footprint patterns. J. Neurosci. Methods 75, 49-54).

Compounds are tested against sham operated and vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Inflammatory Pain

Inflammatory pain is induced mainly in rats by injection of 0.75 mg carrageenan or complete Freund's adjuvant into one hind paw. The animals develop an edema with mechanical allodynia as well as thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy, Paw thermal stimulator, G. Ozaki, University of California, USA). For edema measurement two methods are being used. In the first method, the animals are sacrificed and the affected hindpaws sectioned and weighed. The second method comprises differences in paw volume by measuring water displacement in a plethysmometer (Ugo Basile, Comerio, Italy).

Compounds are tested against uninflamed as well as vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

5 ***Diabetic Neuropathic Pain***

Rats treated with a single intraperitoneal injection of 50 to 80 mg/kg streptozotocin develop a profound hyperglycemia and mechanical allodynia within 1 to 3 weeks. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA).

Compounds are tested against diabetic and non-diabetic vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

2. Parkinson's disease

6-Hydroxydopamine (6-OH-DA) Lesion

Degeneration of the dopaminergic nigrostriatal and striatopallidal pathways is the central pathological event in Parkinson's disease. This disorder has been mimicked experimentally in rats using single/sequential unilateral stereotaxic injections of 6-OH-DA into the medium forebrain bundle (MFB).

Male Wistar rats (Harlan Winkelmann, Germany), weighing 200±250 g at the beginning of the experiment, are used. The rats are maintained in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water when not in experimental sessions. The following in vivo protocols are approved by the governmental authorities. All efforts are made to minimize

animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

5 Animals are administered pargyline on the day of surgery (Sigma, St. Louis, MO, USA; 50 mg/kg i.p.) in order to inhibit metabolism of 6-OHDA by monoamine oxidase and desmethylinipramine HCl (Sigma; 25 mg/kg i.p.) in order to prevent uptake of 6-OHDA by noradrenergic terminals. Thirty minutes later the rats are anesthetized with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. In order to lesion the DA nigrostriatal pathway 4 µl of 0.01% ascorbic acid-saline
10 containing 8 µg of 6-OHDA HBr (Sigma) are injected into the left medial fore-brain bundle at a rate of 1 µl/min (2.4 mm anterior, 1.49 mm lateral, -2.7 mm ventral to Bregma and the skull surface). The needle is left in place an additional 5 min to allow diffusion to occur.

15 Stepping Test

Forelimb akinesia is assessed three weeks following lesion placement using a modified stepping test protocol. In brief, the animals are held by the experimenter with one hand fixing the hindlimbs and slightly raising the hind part above the
20 surface. One paw is touching the table, and is then moved slowly sideways (5 s for 1 m), first in the forehand and then in the backhand direction. The number of adjusting steps is counted for both paws in the backhand and forehand direction of movement. The sequence of testing is right paw forehand and backhand adjusting stepping, followed by left paw forehand and backhand directions. The test is repeated three
25 times on three consecutive days, after an initial training period of three days prior to the first testing. Forehand adjusted stepping reveals no consistent differences between lesioned and healthy control animals. Analysis is therefore restricted to backhand adjusted stepping.

Balance Test

Balance adjustments following postural challenge are also measured during the stepping test sessions. The rats are held in the same position as described in the
5 stepping test and, instead of being moved sideways, tilted by the experimenter towards the side of the paw touching the table. This maneuver results in loss of balance and the ability of the rats to regain balance by forelimb movements is scored on a scale ranging from 0 to 3. Score 0 is given for a normal forelimb placement. When the forelimb movement is delayed but recovery of postural balance detected,
10 score 1 is given. Score 2 represents a clear, yet insufficient, forelimb reaction, as evidenced by muscle contraction, but lack of success in recovering balance, and score 3 is given for no reaction of movement. The test is repeated three times a day on each side for three consecutive days after an initial training period of three days prior to the first testing.

15

Staircase Test (Paw Reaching)

A modified version of the staircase test is used for evaluation of paw reaching behavior three weeks following primary and secondary lesion placement. Plexiglass
20 test boxes with a central platform and a removable staircase on each side are used. The apparatus is designed such that only the paw on the same side at each staircase can be used, thus providing a measure of independent forelimb use. For each test the animals are left in the test boxes for 15 min. The double staircase is filled with 7 x 3 chow pellets (Precision food pellets, formula: P, purified rodent diet, size 45 mg;
25 Sandown Scientific) on each side. After each test the number of pellets eaten (successfully retrieved pellets) and the number of pellets taken (touched but dropped) for each paw and the success rate (pellets eaten/pellets taken) are counted separately. After three days of food deprivation (12 g per animal per day) the animals are tested for 11 days. Full analysis is conducted only for the last five days.

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MPTP treatment

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) causes degeneration of mesencephalic dopaminergic (DAergic) neurons in rodents, non-human primates, and humans and, in so doing, reproduces many of the symptoms of Parkinson's disease. MPTP leads to a marked decrease in the levels of dopamine and its metabolites, and in the number of dopaminergic terminals in the striatum as well as severe loss of the tyrosine hydroxylase (TH)-immunoreactive cell bodies in the substantia nigra, pars compacta.

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In order to obtain severe and long-lasting lesions, and to reduce mortality, animals receive single injections of MPTP, and are then tested for severity of lesion 7–10 days later. Successive MPTP injections are administered on days 1, 2 and 3. Animals receive application of 4 mg/kg MPTP hydrochloride (Sigma) in saline once daily. All injections are intraperitoneal (i.p.) and the MPTP stock solution is frozen between injections. Animals are decapitated on day 11.

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Immunohistology

At the completion of behavioral experiments, all animals are anaesthetized with 3 ml thiopental (1 g/40 ml i.p., Tyrol Pharma). The mice are perfused transcardially with 0.01 M PBS (pH 7.4) for 2 min, followed by 4% paraformaldehyde (Merck) in PBS for 15 min. The brains are removed and placed in 4% paraformaldehyde for 24 h at 4 °C. For dehydration they are then transferred to a 20% sucrose (Merck) solution in 0.1 M PBS at 4 °C until they sink. The brains are frozen in methylbutan at -20 °C for 2 min and stored at -70 °C. Using a sledge microtome (mod. 3800-Frigocut, Leica), 25 µm sections are taken from the genu of the corpus callosum (AP 1.7 mm) to the hippocampus (AP 21.8 mm) and from AP 24.16 to AP 26.72. Forty-six sections are cut and stored in sorters in 0.25 M Tris buffer (pH 7.4) for immunohistochemistry.

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A series of sections is processed for free-floating tyrosine hydroxylase (TH) immunohistochemistry. Following three rinses in 0.1 M PBS, endogenous peroxidase activity is quenched for 10 min in 0.3% H₂O₂ ±PBS. After rinsing in PBS, sections are preincubated in 10% normal bovine serum (Sigma) for 5 min as blocking agent and transferred to either primary anti-rat TH rabbit antiserum (dilution 1:2000).

Following overnight incubation at room temperature, sections for TH immunoreactivity are rinsed in PBS (2 x10 min) and incubated in biotinylated anti-rabbit immunoglobulin G raised in goat (dilution 1:200) (Vector) for 90 min, rinsed repeatedly and transferred to Vectastain ABC (Vector) solution for 1 h. 3,3'-Diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.1 M PBS, supplemented with 0.005% H₂O₂ , serves as chromogen in the subsequent visualization reaction. Sections are mounted on to gelatin-coated slides, left to dry overnight, counter-stained with hematoxylin dehydrated in ascending alcohol concentrations and cleared in butylacetate. Coverslips are mounted on entellan.

Rotarod Test

We use a modification of the procedure described by Rozas and Labandeira-Garcia (1997), with a CR-1 Rotamex system (Columbus Instruments, Columbus, OH) comprising an IBM-compatible personal computer, a CIO-24 data acquisition card, a control unit, and a four-lane rotarod unit. The rotarod unit consists of a rotating spindle (diameter 7.3 cm) and individual compartments for each mouse. The system software allows preprogramming of session protocols with varying rotational speeds (0–80 rpm). Infrared beams are used to detect when a mouse has fallen onto the base grid beneath the rotarod. The system logs the fall as the end of the experiment for that mouse, and the total time on the rotarod, as well as the time of the fall and all the set-up parameters, are recorded. The system also allows a weak current to be passed through the base grid, to aid training.

3. Dementia

The object recognition task

5 The object recognition task has been designed to assess the effects of experimental manipulations on the cognitive performance of rodents. A rat is placed in an open field, in which two identical objects are present. The rats inspects both objects during the first trial of the object recognition task. In a second trial, after a retention interval of for example 24 hours, one of the two objects used in the first trial, the 'familiar' object, and a novel object are placed in the open field. The inspection time at each of
10 the objects is registered. The basic measures in the OR task is the time spent by a rat exploring the two object the second trial. Good retention is reflected by higher exploration times towards the novel than the 'familiar' object.

Administration of the putative cognition enhancer prior to the first trial
15 predominantly allows assessment of the effects on acquisition, and eventually on consolidation processes. Administration of the testing compound after the first trial allows to assess the effects on consolidation processes, whereas administration before the second trial allows to measure effects on retrieval processes.

20 The passive avoidance task

The passive avoidance task assesses memory performance in rats and mice. The inhibitory avoidance apparatus consists of a two-compartment box with a light compartment and a dark compartment. The two compartments are separated by a
25 guillotine door that can be operated by the experimenter. A threshold of 2 cm separates the two compartments when the guillotine door is raised. When the door is open, the illumination in the dark compartment is about 2 lux. The light intensity is about 500 lux at the center of the floor of the light compartment.

30 Two habituation sessions, one shock session, and a retention session are given, separated by inter-session intervals of 24 hours. In the habituation sessions and the

retention session the rat is allowed to explore the apparatus for 300 sec. The rat is placed in the light compartment, facing the wall opposite to the guillotine door. After an accommodation period of 15 sec. the guillotine door is opened so that all parts of the apparatus can be visited freely. Rats normally avoid brightly lit areas and will enter the dark compartment within a few seconds.

In the shock session the guillotine door between the compartments is lowered as soon as the rat has entered the dark compartment with its four paws, and a scrambled 1 mA footshock is administered for 2 sec. The rat is removed from the apparatus and put back into its home cage. The procedure during the retention session is identical to that of the habituation sessions.

The step-through latency, that is the first latency of entering the dark compartment (in sec.) during the retention session is an index of the memory performance of the animal; the longer the latency to enter the dark compartment, the better the retention is. A testing compound is given half an hour before the shock session, together with 1 mg*kg⁻¹ scopolamine. Scopolamine impairs the memory performance during the retention session 24 hours later. If the test compound increases the enter latency compared with the scopolamine-treated controls, is likely to possess cognition enhancing potential.

The Morris water escape task

The Morris water escape task measures spatial orientation learning in rodents. It is a test system that has extensively been used to investigate the effects of putative therapeutic on the cognitive functions of rats and mice. The performance of an animal is assessed in a circular water tank with an escape platform that is submerged about 1 cm below the surface of the water. The escape platform is not visible for an animal swimming in the water tank. Abundant extra-maze cues are provided by the furniture in the room, including desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that is playing softly.

The animals receive four trials during five daily acquisition sessions. A trial is started by placing an animal into the pool, facing the wall of the tank. Each of four starting positions in the quadrants north, east, south, and west is used once in a series of four trials; their order is randomized. The escape platform is always in the same position. A trial is terminated as soon as the animal had climbs onto the escape platform or when 90 seconds have elapsed, whichever event occurs first. The animal is allowed to stay on the platform for 30 seconds. Then it is taken from the platform and the next trial is started. If an animal did not find the platform within 90 seconds it is put on the platform by the experimenter and is allowed to stay there for 30 seconds. After the fourth trial of the fifth daily session, an additional trial is given as a probe trial: the platform is removed, and the time the animal spends in the four quadrants is measured for 30 or 60 seconds. In the probe trial, all animals start from the same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

Four different measures are taken to evaluate the performance of an animal during acquisition training: escape latency, traveled distance, distance to platform, and swimming speed. The following measures are evaluated for the probe trial: time (s) in quadrants and traveled distance (cm) in the four quadrants. The probe trial provides additional information about how well an animal learned the position of the escape platform. If an animal spends more time and swims a longer distance in the quadrant where the platform had been positioned during the acquisition sessions than in any other quadrant, one concludes that the platform position has been learned well.

In order to assess the effects of putative cognition enhancing compounds, rats or mice with specific brain lesions which impair cognitive functions, or animals treated with compounds such as scopolamine or MK-801, which interfere with normal learning, or aged animals which suffer from cognitive deficits, are used.

The T-maze spontaneous alternation task

The T-maze spontaneous alternation task (TeMCAT) assesses the spatial memory performance in mice. The start arm and the two goal arms of the T-maze are provided with guillotine doors which can be operated manually by the experimenter. A mouse is put into the start arm at the beginning of training. The guillotine door is closed. In the first trial, the 'forced trial', either the left or right goal arm is blocked by lowering the guillotine door. After the mouse has been released from the start arm, it will negotiate the maze, eventually enter the open goal arm, and return to the start position, where it will be confined for 5 seconds, by lowering the guillotine door. Then, the animal can choose freely between the left and right goal arm (all guillotine-doors opened) during 14 'free choice' trials. As soon as the mouse has entered one goal arm, the other one is closed. The mouse eventually returns to the start arm and is free to visit whichever goal arm it wants after having been confined to the start arm for 5 seconds. After completion of 14 free choice trials in one session, the animal is removed from the maze. During training, the animal is never handled.

The percent alternations out of 14 trials is calculated. This percentage and the total time needed to complete the first forced trial and the subsequent 14 free choice trials (in s) is analyzed. Cognitive deficits are usually induced by an injection of scopolamine, 30 min before the start of the training session. Scopolamine reduced the per-cent alternations to chance level, or below. A cognition enhancer, which is always administered before the training session, will at least partially, antagonize the scopolamine-induced reduction in the spontaneous alternation rate.

EXAMPLE 8

Proliferation inhibition assay: Antisense oligonucleotides suppress the growth of cancer cell lines

5 The cell line used for testing is the human colon cancer cell line HCT116. Cells are cultured in RPMI-1640 with 10-15% fetal calf serum at a concentration of 10,000 cells per milliliter in a volume of 0.5 ml and kept at 37 °C in a 95% air/5%CO₂ atmosphere.

10 Phosphorothioate oligoribonucleotides are synthesized on an Applied Biosystems Model 380B DNA synthesizer using phosphoroamidite chemistry. A sequence of 24 bases complementary to the nucleotides at position 1 to 24 of SEQ ID NO:1 is used as the test oligonucleotide. As a control, another (random) sequence is used: 5'-TCA
15 ACT GAC TAG ATG TAC ATG GAC-3'. Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate buffered saline at the desired concentration. Purity of the oligonucleotides is tested by capillary gel electrophoresis and ion exchange HPLC. The purified
oligonucleotides are added to the culture medium at a concentration of 10 µM once per day for seven days.

20 The addition of the test oligonucleotide for seven days results in significantly reduced expression of human serine/threonine protein kinase as determined by Western blotting. This effect is not observed with the control oligonucleotide. After 3 to 7 days, the number of cells in the cultures is counted using an automatic cell
25 counter. The number of cells in cultures treated with the test oligonucleotide (expressed as 100%) is compared with the number of cells in cultures treated with the control oligonucleotide. The number of cells in cultures treated with the test oligonucleotide is not more than 30% of control, indicating that the inhibition of
human serine/threonine protein kinase has an anti-proliferative effect on cancer cells.

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EXAMPLE 9***In vivo testing of compounds/target validation*****1. Acute Mechanistic Assays*****1.1. Reduction in Mitogenic Plasma Hormone Levels***

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This non-tumor assay measures the ability of a compound to reduce either the endogenous level of a circulating hormone or the level of hormone produced in response to a biologic stimulus. Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.). At a predetermined time after administration of test compound, blood plasma is collected. Plasma is assayed for levels of the hormone of interest. If the normal circulating levels of the hormone are too low and/or variable to provide consistent results, the level of the hormone may be elevated by a pre-treatment with a biologic stimulus (i.e., LHRH may be injected i.m. into mice at a dosage of 30 ng/mouse to induce a burst of testosterone synthesis). The timing of plasma collection would be adjusted to coincide with the peak of the induced hormone response. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value \leq 0.05 compared to the vehicle control group.

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1.2. Hollow Fiber Mechanism of Action Assay

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Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol, these may include assays for gene expression (bDNA, PCR, or Taqman), or a specific biochemical activity (i.e., cAMP levels. Results are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at p \leq 0.05 as compared to the vehicle control group.

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2. Subacute Functional *In Vivo* Assays

2.1. *Reduction in Mass of Hormone Dependent Tissues*

This is another non-tumor assay that measures the ability of a compound to reduce the mass of a hormone dependent tissue (i.e., seminal vesicles in males and uteri in females). Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.) according to a predetermined schedule and for a predetermined duration (i.e., 1 week). At termination of the study, animals are weighed, the target organ is excised, any fluid is expressed, and the weight of the organ is recorded. Blood plasma may also be collected. Plasma may be assayed for levels of a hormone of interest or for levels of test agent. Organ weights may be directly compared or they may be normalized for the body weight of the animal. Compound effects are compared to a vehicle-treated control group. An F-test is performed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value ≤ 0.05 compared to the vehicle control group.

2.2. *Hollow Fiber Proliferation Assay*

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol. Cell proliferation is determined by measuring a marker of cell number (i.e., MTT or LDH). The cell number and change in cell number from the starting inoculum are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at p ≤ 0.05 as compared to the vehicle control group.

2.3. *Anti-angiogenesis Models*

2.3.1. *Corneal Angiogenesis*

Hydron pellets with or without growth factors or cells are implanted into a micropocket surgically created in the rodent cornea. Compound administration may be systemic or local (compound mixed with growth factors in the hydron pellet). Corneas are harvested at 7 days post implantation immediately following intracardiac infusion of colloidal carbon and are fixed in 10% formalin. Readout is qualitative scoring and/or image analysis. Qualitative scores are compared by Rank Sum test. Image analysis data is evaluated by measuring the area of neovascularization (in pixels) and group averages are compared by Student's t-test (2 tail). Significance is $p \leq 0.05$ as compared to the growth factor or cells only group.

2.3.2. *Matrigel Angiogenesis*

Matrigel, containing cells or growth factors, is injected subcutaneously. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Matrigel plugs are harvested at predetermined time point(s) and prepared for readout. Readout is an ELISA-based assay for hemoglobin concentration and/or histological examination (i.e. vessel count, special staining for endothelial surface markers: CD31, factor-8). Readouts are analyzed by Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$ as compared to the vehicle control group.

25 3. **Primary Antitumor Efficacy**

3.1. *Early Therapy Models*

3.1.1. *Subcutaneous Tumor*

Tumor cells or fragments are implanted subcutaneously on Day 0. Vehicle and/or compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting at a time, usually on Day 1, prior to the ability to measure the tumor

burden. Body weights and tumor measurements are recorded 2-3 times weekly. Mean net body and tumor weights are calculated for each data collection day. Anti-tumor efficacy may be initially determined by comparing the size of treated (T) and control (C) tumors on a given day by a Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$. The experiment may also be continued past the end of dosing in which case tumor measurements would continue to be recorded to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \leq 0.05$.

3.1.2. *Intraperitoneal/Intracranial Tumor Models*

Tumor cells are injected intraperitoneally or intracranially on Day 0. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting on Day 1. Observations of morbidity and/or mortality are recorded twice daily. Body weights are measured and recorded twice weekly. Morbidity/mortality data is expressed in terms of the median time of survival and the number of long-term survivors is indicated separately. Survival times are used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment.

3.2. *Established Disease Model*

Tumor cells or fragments are implanted subcutaneously and grown to the desired size for treatment to begin. Once at the predetermined size range, mice are randomized into treatment groups. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and

recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \text{ value} \leq 0.05$ compared to the vehicle control group.

3.3. *Orthotopic Disease Models*

3.3.1. *Mammary Fat Pad Assay*

Tumor cells or fragments, of mammary adenocarcinoma origin, are implanted directly into a surgically exposed and reflected mammary fat pad in rodents. The fat pad is placed back in its original position and the surgical site is closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group.

Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by

generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \text{ value} \leq 0.05$ compared to the vehicle control group. In addition, this model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ, or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.2. *Intraprostatic Assay*

Tumor cells or fragments, of prostatic adenocarcinoma origin, are implanted directly into a surgically exposed dorsal lobe of the prostate in rodents. The prostate is externalized through an abdominal incision so that the tumor can be implanted specifically in the dorsal lobe while verifying that the implant does not enter the seminal vesicles. The successfully inoculated prostate is replaced in the abdomen and the incisions through the abdomen and skin are closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is performed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the lungs), or measuring the target organ weight (i.e., the regional lymph nodes). The means of these endpoints are compared by Student's t-test after conducting an F-test,

with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.3. *Intrabronchial Assay*

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Tumor cells of pulmonary origin may be implanted intrabronchially by making an incision through the skin and exposing the trachea. The trachea is pierced with the beveled end of a 25 gauge needle and the tumor cells are inoculated into the main bronchus using a flat-ended 27 gauge needle with a 90° bend. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is performed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the contralateral lung), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

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3.3.4. *Intracecal Assay*

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Tumor cells of gastrointestinal origin may be implanted intracecally by making an abdominal incision through the skin and externalizing the intestine. Tumor cells are inoculated into the cecal wall without penetrating the lumen of the intestine using a 27 or 30 gauge needle. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using

either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the liver), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

4. Secondary (Metastatic) Antitumor Efficacy

4.1. Spontaneous Metastasis

Tumor cells are inoculated s.c. and the tumors allowed to grow to a predetermined range for spontaneous metastasis studies to the lung or liver. These primary tumors are then excised. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule which may include the period leading up to the excision of the primary tumor to evaluate therapies directed at inhibiting the early stages of tumor metastasis. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment for both of these endpoints.

4.2. *Forced Metastasis*

Tumor cells are injected into the tail vein, portal vein, or the left ventricle of the heart in experimental (forced) lung, liver, and bone metastasis studies, respectively. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance at $p \leq 0.05$ compared to the vehicle control group in the experiment for both endpoints.

EXAMPLE 10

Diabetes: In vivo testing of compounds/target validation

1. Glucose Production:

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight fasted normal rats or mice have elevated rates of gluconeogenesis as do streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose

production will decrease plasma glucose levels compared to the vehicle-treated control group.

2. Insulin Sensitivity:

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Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma glucose and insulin levels when compared to the vehicle-treated control group.

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3. Insulin Secretion:

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Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

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Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, test compounds which regulate serine/threonine protein kinase are administered by different routes (p.o., i.p., s.c., or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Test compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60, and 90 minutes and plasma glucose levels determined. Test compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

4. Glucose Production:

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight fasted normal rats or mice have elevated rates of gluconeogenesis as do streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose production will decrease plasma glucose levels compared to the vehicle-treated control group.

5. Insulin Sensitivity:

Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma glucose and insulin levels when compared to the vehicle-treated control group.

6. Insulin Secretion:

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

EXAMPLE 11

Quantitative RT-PCR analysis of cancer tissues and obesity and diabetes tissues.

RNA extraction and cDNA preparation

5 Total RNA used for Taqman quantitative analysis were either purchased (Clontech, CA) or extracted from tissues using TRIzol reagent (Life Technologies, MD) according to a modified vendor protocol which utilizes the Rneasy protocol (Qiagen, CA).

10 One hundred µg of each RNA were treated with DNase I using RNase free- DNase (Qiagen, CA) for use with RNeasy or QiaAmp columns.

After elution and quantitation with Ribogreen (Molecular Probes Inc., OR) each sample was reverse transcribed using the GibcoBRL Superscript II First Strand
15 Synthesis System for RT-PCR according to vendor protocol (Life Technologies, MD). The final concentration of RNA in the reaction mix was 50ng/µL. Reverse transcription was performed with 0.5µg of Oligo dT primer for the cancer panel and 50ng of Random Hexamers for the obesity and diabetes panel.

20 *TaqMan quantitative analysis*

Specific primers and probe were designed according to PE Applied Biosystems recommendations and are listed below:

forward primer: 5'-(GACAGCTCCCAGTCCGAGAA)-3' (SEQ ID NO:9)

25 reverse primer: 5'-(TCCTGGCTCCTCTTCATCTTTT)-3' (SEQ ID NO:10)

probe: SYBR Green

The expected length of the PCR product was 100 bp.

Quantitation experiments were performed on 25 ng of reverse transcribed RNA from each sample. Each determination was done in duplicate. 18S ribosomal RNA was
30 measured as a control using the Pre-Developed TaqMan Assay Reagents (PDAR)(PE Applied Biosystems, CA). Assay reaction mix was as follows:

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		final
	TaqMan SYBR Green PCR Master Mix (2x)	1x
	(PE Applied Biosystems, CA)	
	Forward primer (SEQ ID NO:9)	300nM
5	Reverse primer (SEQ ID NO:10)	300nM
	cDNA	25ng
	Water to 25µL	
	18s control:	
10	Taqman Universal PCR Master Mix (2x)	1x
	(PE Applied Biosystems, CA)	
	PDAR control – 18S RNA (20x)	1x
	18S ribosomal forward primer	300nM
	18S ribosomal reverse primer	300nM
15	cDNA	25ng
	Water to 25µl	
	PCR conditions:	
	Once: 2 minutes at 50 °C	
20	10 minutes at 95 °C	
	40cycles: 15 sec. at 95 °C	
	1 minute at 60 °C	

25 The experiment was performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR were processed as described in the ABI Prism 7700 user's manual.

For cancer tissues, fold change was calculated using the delta-delta C_T method with normalization to the 18S RNA values. Results are shown in Fig. 13.

For obesity and diabetes tissues, relative expression was determined as follows. Ct values were normalized to 18S RNA values. The highest expressing tissue was then assigned a value of 100. Expression levels for the remaining tissues were then expressed as percentages of the highest expressing tissue ($[\Delta C_T \text{ of tissue } x / \Delta C_T \text{ of highest expresser}] \times 100$). Results are shown in Figs. 14a and b.

EXAMPLE 12

Treatment of asthma with a reagent that specifically binds to a serine-threonine protein kinase activity gene product

10

Synthesis of antisense serine-threonine protein kinase activity oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoroamidite procedure (Uhlmann *et al.*, *Chem. Rev.* 90, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.)* 105, 361-362, 1953).

20

An aqueous composition containing the antisense oligonucleotides is administered to the patient by inhalation.

25

Severity of asthma is monitored over a period of days or weeks by noting changes in patients' asthmatic symptoms, measuring lung function, or measuring changes in markers of lung inflammation such as numbers of inflammatory cells or concentrations of inflammatory mediators in fluid sampled from patients' lungs by bronchoalveolar lavage. Asthma severity is reduced due to decreased serine-threonine protein kinase activity.

30

EXAMPLE 13*Asthma: In vivo validation of novel compounds*

1. Tests for activity of T cells are used to evaluate agents that modulate the expression or activity of costimulatory molecules-cytokines, cytokine
5 receptors, signalling molecules, or other molecules involved in T cell activation

Mouse anti-CD3-induced cytokine production model:

- 10 BALB/c mice are injected with a single intravenous injection of 10 µg of 145-2C11 (purified hamster anti-mouse CD3 monoclonal antibodies, PHARMINGEN). Compound is administered intraperitoneally 60 min prior to the anti-CD3 mAb injection. Blood is collected 90 min after the antibody injection. Serum is obtained by centrifugation at 3000 r.p.m. for 10 min.
15 Serum levels of cytokines, such as IL-2 and IL-4, or other secreted molecules are determined by an ELISA. Proteins which regulate the CD3 downstream signaling can be evaluated in this model.

2. Tests for activity of B cells are used to evaluate agents that modulate the
20 expression or activity of the B cell receptor, signaling molecules, or other molecules involved in B cell activation/immunoglobulin class switching

Mouse anti-IgD induced IgE production model:

- 25 BALB/c mice are injected intravenously with 0.8 mg of purified goat anti-mouse IgD antibody or PBS (defined as day 0). Compound is administered intraperitoneally from day 0 to day 6. On day 7 blood is collected and serum is obtained by centrifugation at 3000 r.p.m. for 10 min. Serum levels of total IgE are determined by YAMASA's ELISA kit and other Ig subtypes are
30 measured by an Ig ELISA KIT (Rougier Bio-tech's, Montreal, Canada).

Proteins that regulate IgD downstream signaling and Ig class switching can be evaluated.

3. Tests for activity of monocytes/macrophages are used to evaluate agents that modulate the expression or activity of signalling molecules, transcription factors.

Mouse LPS-induced TNF- α production model:

Compound is administered to BALB/c mice by intraperitoneal injection and one hour later the mice given LPS (200 μ g/mouse) by intraperitoneal injection. Blood is collected 90 minutes after the LPS injection and plasma is obtained. TNF- α concentration in the sample is determined using an ELISA kit. Proteins that regulate downstream effects of LPS stimulation, such as NF- κ B activation, can be evaluated.

4. Tests for activity of eosinophils are used to evaluate agents that modulate the expression or activity of the eotaxin receptor, signaling molecules, cytoskeletal molecules, or adhesion molecules.

Mouse eotaxin-induced eosinophilia model:

BALB/c mice are injected intradermally with a 2.5 ml of air on days -6 and -3 to prepare an airpouch. On day 0, compound is administered intraperitoneally, and 30 minutes later, IL-5 (300 ng/mouse) is injected intravenously. After an additional 30 minutes, eotaxin is injected (3 μ g/mouse, i.d.). Four hours after the eotaxin injection, leukocytes in the airpouch exudate are collected and the number of total cells is counted. Differential cell counts in the exudate are performed by staining with May-Grunwald Gimsa solution. Proteins that regulate signaling by the eotaxin receptor or regulate eosinophil trafficking can be evaluated.

5. Passive cutaneous anaphylaxis (PCA) test in rats 6 Weeks old male Wistar rats are sensitized intradermally (i.d.) on their shaved backs with 50 μ l of 0.1 μ g/ml mouse anti-DNP IgE monoclonal antibody (SPE-7) under a light anesthesia. After 24 hours, the rats are challenged intravenously with 1 ml of

saline containing 0.6 mg DNP-BSA (30) (LSL CO., LTD) and 0.005 g of Evans blue. Compounds are injected intraperitoneally (i.p.) 0.5 hr prior to antigen injection. Rats without the sensitization, challenge, and compound treatment are used as a control and rats with sensitization, challenge and vehicle treatment are used to determine the value without inhibition. Thirty minutes after the challenge, the rats are sacrificed, and the skin of the back is removed. Evans blue dye in the skin is extracted in formamide overnight at 63°C. Absorbance at 620 nm is then measured to obtain the optical density of the leaked dye.

Percent inhibition of PCA with a compound is calculated as follows:

$$\% \text{ inhibition} = \{(\text{mean vehicle value} - \text{sample value}) / (\text{mean vehicle value} - \text{mean control value})\} \times 100$$

Proteins that regulate mast cell degranulation, vascular permeability, or receptor antagonists against histamine receptors, serotonin receptors, or cysteinyl leukotriene receptors can be evaluated.

6. Anaphylactic bronchoconstriction in rats

6 Weeks old male Wistar rats are sensitized intravenously (i.v.) with 10 µg mouse anti-DNP IgE, SPE-7, and 1 days later, the rats are challenged intravenously with 0.3 ml of saline containing 1.5 mg DNP-BSA (30) under anesthesia with urethane (1000 mg/kg, i.p.) and gallamine (50 mg/kg, i.v.). The trachea is cannulated for artificial respiration (2 ml/stroke, 70 strokes/min). Pulmonary inflation pressure (PIP) is recorded through a side-arm of the cannula connected to a pressure transducer. Changes in PIP reflect a change of both resistance and compliance of the lungs. To evaluate a compound, the compound is given i.v. 5 min before challenge.

Proteins that regulate mast cell degranulation, vascular permeability or receptor antagonists against histamine receptors, serotonin receptors, or

cysteinyl leukotriene receptors can be evaluated. Proteins that regulate the contraction of smooth muscle can be also evaluated.

7. *T cell adhesion to smooth muscle cells or endothelial cells*

5 A purified population of T cells is prepared by ficoll density centrifugation followed by separation on a nylon wool column, rosetting with sheep red blood cells, or using magnetic beads coated with antibodies. The T cells are activated with mitogen for 36 to 42 hours and labeled with ^3H -thymidine during the last 16 hours of the activation. Airway smooth muscle cells or
10 bronchial microvascular endothelial cells are obtained from lung transplant tissue, from bronchus resections from cancer patients, from cadavers, or as cell lines from commercial sources. If fresh tissue is used as the source of cells, the smooth muscle cells and endothelial cells can be isolated from tissue by dissection followed by digestion for 30-60 minutes in a solution containing
15 1.7 mM ethyleneglycol-bis-(beta-aminoethylether)-N,N,N',N'-tetraacetic acid, 640 U/ml collagenase, 10 mg/ml soybean trypsin inhibitor, and 10 U/ml elastase. The smooth muscle cells or endothelial cells are grown in 24-well tissue culture dishes until confluent and then treated with a test compound and inflammatory mediators, such as $\text{TNF-}\alpha$ for 24 hours. To measure adhesion,
20 6×10^5 T cells are added per well and allowed to adhere for one hour at 37°C . Nonadherent cells are removed by washing six times gently with medium. Finally, the remaining adherent cells are lysed by adding 300 μl 1% Triton-X 100 in PBS to each well and quantitating the radioactivity in a scintillation counter. The percent binding is calculated as counts recovered
25 from adherent cells/total input counts $\times 100\%$

CLAIMS

1. An isolated polynucleotide encoding a serine-threonine protein kinase polypeptide and being selected from the group consisting of:
 - 5 a) a polynucleotide encoding a serine-threonine protein kinase polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 93% identical to the amino acid sequence shown in SEQ ID NO: 2; and

10 the amino acid sequence shown in SEQ ID NO: 2.
 - b) a polynucleotide comprising the sequence of SEQ ID NO: 1;
 - c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);
 - 15 d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and
 - e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a to (d).
- 20 2. An expression vector containing any polynucleotide of claim 1.
3. A host cell containing the expression vector of claim 2.
4. A substantially purified serine-threonine protein kinase polypeptide encoded
25 by a polynucleotide of claim 1.
5. A method for producing a serine-threonine protein kinase polypeptide, wherein the method comprises the following steps:
 - 30 a) culturing the host cell of claim 3 under conditions suitable for the expression of the serine-threonine protein kinase polypeptide; and

- b) recovering the serine-threonine protein kinase polypeptide from the host cell culture.
6. A method for detection of a polynucleotide encoding a serine-threonine protein kinase polypeptide in a biological sample comprising the following steps:
- 5 a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and
- b) detecting said hybridization complex.
- 10 7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.
8. A method for the detection of a polynucleotide of claim 1 or a serine-threonine protein kinase polypeptide of claim 4 comprising the steps of:
- 15 contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the serine-threonine protein kinase polypeptide.
9. A diagnostic kit for conducting the method of any one of claims 6 to 8.
- 20 10. A method of screening for agents which decrease the activity of a serine-threonine protein kinase, comprising the steps of:
- contacting a test compound with any serine-threonine protein kinase polypeptide encoded by any polynucleotide of claim 1;
- 25 detecting binding of the test compound to the serine-threonine protein kinase polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a serine-threonine protein kinase.
- 30 11. A method of screening for agents which regulate the activity of a serine-threonine protein kinase, comprising the steps of:

contacting a test compound with a serine-threonine protein kinase polypeptide encoded by any polynucleotide of claim 1; and
detecting a serine-threonine protein kinase activity of the polypeptide, wherein a test compound which increases the serine-threonine protein kinase activity is identified as a potential therapeutic agent for increasing the activity of the serine-threonine protein kinase, and wherein a test compound which decreases the serine-threonine protein kinase activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the serine-threonine protein kinase.

10

12. A method of screening for agents which decrease the activity of a serine-threonine protein kinase, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of serine-threonine protein kinase.

15

13. A method of reducing the activity of serine-threonine protein kinase, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any serine-threonine protein kinase polypeptide of claim 4, whereby the activity of serine-threonine protein kinase is reduced.

20

14. A reagent that modulates the activity of a serine-threonine protein kinase polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

25

15. A pharmaceutical composition, comprising:
the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

30

16. Use of the expression vector of claim 2 or the reagent of claim 14 for the preparation of a medicament for modulating the activity of a serine-threonine protein kinase in a disease.
- 5 17. Use of claim 16 wherein the disease is cancer, a CNS disorder, obesity, diabetes, asthma, or COPD.
18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.
- 10 19. The cDNA of claim 18 which comprises SEQ ID NO:1.
20. The cDNA of claim 18 which consists of SEQ ID NO:1.
- 15 21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.
22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO:1.
- 20 23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.
24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO:1.
- 25 25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.
- 30 26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO:2.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO:2.
- 5 28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
isolating the polypeptide.
- 10 29. The method of claim 28 wherein the expression vector comprises SEQ ID NO:1.
- 15 30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
detecting the hybridization complex.
- 20 31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.
- 25 32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:
a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1; and
instructions for the method of claim 30.
- 30 33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and detecting the reagent-polypeptide complex.

- 5 34. The method of claim 33 wherein the reagent is an antibody.
35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:
an antibody which specifically binds to the polypeptide; and
10 instructions for the method of claim 33.
36. A method of screening for agents which can modulate the activity of a human serine-threonine protein kinase, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid
15 sequence selected from the group consisting of: (1) amino acid sequences which are at least about 93% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and
detecting binding of the test compound to the polypeptide, wherein a test
20 compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human serine-threonine protein kinase.
37. The method of claim 36 wherein the step of contacting is in a cell.
38. The method of claim 36 wherein the cell is *in vitro*.
- 25 39. The method of claim 36 wherein the step of contacting is in a cell-free system.
40. The method of claim 36 wherein the polypeptide comprises a detectable label.
- 30

41. The method of claim 36 wherein the test compound comprises a detectable label.
42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.
43. The method of claim 36 wherein the polypeptide is bound to a solid support.
44. The method of claim 36 wherein the test compound is bound to a solid support.
45. A method of screening for agents which modulate an activity of a human serine-threonine protein kinase, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 93% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human serine-threonine protein kinase, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human serine-threonine protein kinase.
46. The method of claim 45 wherein the step of contacting is in a cell.
47. The method of claim 45 wherein the cell is *in vitro*.
48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human serine-threonine protein kinase, comprising the steps of:
contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1; and
5 detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human serine-threonine protein kinase.
50. The method of claim 49 wherein the product is a polypeptide.
- 10 51. The method of claim 49 wherein the product is RNA.
52. A method of reducing activity of a human serine-threonine protein kinase, comprising the step of:
15 contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1, whereby the activity of a human serine-threonine protein kinase is reduced.
- 20 53. The method of claim 52 wherein the product is a polypeptide.
54. The method of claim 53 wherein the reagent is an antibody.
55. The method of claim 52 wherein the product is RNA.
- 25 56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.
57. The method of claim 56 wherein the reagent is a ribozyme.
- 30 58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.
60. A pharmaceutical composition, comprising:
a reagent which specifically binds to a polypeptide comprising the amino acid
sequence shown in SEQ ID NO:2; and
a pharmaceutically acceptable carrier.
61. The pharmaceutical composition of claim 60 wherein the reagent is an
antibody.
62. A pharmaceutical composition, comprising:
a reagent which specifically binds to a product of a polynucleotide
comprising the nucleotide sequence shown in SEQ ID NO:1; and
a pharmaceutically acceptable carrier.
63. The pharmaceutical composition of claim 62 wherein the reagent is a
ribozyme.
64. The pharmaceutical composition of claim 62 wherein the reagent is an
antisense oligonucleotide.
65. The pharmaceutical composition of claim 62 wherein the reagent is an
antibody.
66. A pharmaceutical composition, comprising:
an expression vector encoding a polypeptide comprising the amino acid
sequence shown in SEQ ID NO:2; and
a pharmaceutically acceptable carrier.
67. The pharmaceutical composition of claim 66 wherein the expression vector
comprises SEQ ID NO:1.

68. A method of treating a serine-threonine protein kinase dysfunction related disease, wherein the disease is selected from cancer, a CNS disorder, obesity, diabetes, asthma or COPD comprising the step of:
- 5 administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human serine-threonine protein kinase, whereby symptoms of the serine-threonine protein kinase dysfunction related disease are ameliorated.
- 10 69. The method of claim 68 wherein the reagent is identified by the method of claim 36.
70. The method of claim 68 wherein the reagent is identified by the method of claim 45.
- 15 71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

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Fig. 1

caggtgtgca	ttgtgcagaa	gcgggacacg	gagaagatgt
acgccatgaa	gtacatgaac	aagcagcagt	gcatcgagcg
cgacgaggtc	cgcaacgtct	tccgggagct	ggagatcctg
caggagatcg	agcacgtctt	cctgggtgaac	ctctgggtact
ccttccagga	cgaggaggac	atgttcatgg	tcgtggacct
gctactgggc	ggggacctgc	gctaccacct	gcagcagaac
gtgcagttct	ccgaggacac	ggtgaggctg	tacatctgcg
agatggcact	ggctctggac	tacctgcgcg	gccagcacat
catccacaga	gatgtcaagc	ctgacaacat	tctcctggat
gagagaggac	atgcacacct	gaccgacttc	aacattgcca
ccatcatcaa	ggacggggag	cgggcgacgg	cattagcagg
caccaagccg	tacatggctc	cggagatctt	ccactctttt
gtcaacggcg	ggaccggcta	ctccttcgag	gtggactggg
ggtcgggtggg	ggtgatggcc	tatgagctgc	tgcgaggatg
gaggccctat	gacatccact	ccagcaacgc	cgtggagtcc
ctggtgcagc	tgttcagcac	cgtgagcgtc	cagtatgtcc
ccacgtggtc	caaggagatg	gtggccttgc	tgcggaagct
cctcactgtg	aacccccgagc	accggctctc	cagcctccag
gacgtgcagg	cagccccggc	gctggccggc	gtgctgtggg
accacctgag	cgagaagagg	gtggagccgg	gcttcgtgcc
caacaaaggc	cgtctgcact	gcgacccac	ctttgagctg
gaggagatga	tcctggagtc	caggcccctg	cacaagaaga
agaagcgtct	ggccaagaac	aagtcccggg	acaacagcag
ggacagctcc	cagtccgaga	atgactatct	tcaagactgc
ctcgatgcca	tccagcaaga	cttcgtgatt	tttaacagag
aaaagatgaa	gaggagccag	gacctcccga	gggagcctct
ccccgcccct	gagtccaggg	atgctgcgga	gcctgtggag
gacgaggcgg	aa		

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Fig. 2

QVCIVQKRDT EKMYAMKYMN KQQCIERDEV RNVFRELEIL
 QEIEHVFLVN LWYSFQDEED MFMVVDLLLG GDLRYHLQQN
 VQFSEDTVRL YICEMALALD YLRGQHIIHR DVKPDNILLD
 ERGHAHLTDF NIATIIKDGE RATALAGTKP YMAPEIFHSF
 VNGGTGYSFE VDWWSVGVM YELLRGWRPY DIHSSNAVES
 LVQLFSTVSV QYVPTWSKEM VALLRKLLTV NPEHRLSSLQ
 DVQAAPALAG VLWDHLSEKR VEPGFVPNKG RLHCDPTFEL
 EEMILESRLPL HKKKKRLAKN KSRDNSRDSS QSENDYLQDC
 LDAIQQDFVI FNREKMKRSQ DLPREPLPAP ESRDAAEPVE DEAE

Fig. 3

MRSGAERRGSSAAAPPSSPPPGRARPAGSEVSPALPPPAASQPRARDAGDARA
 QPRPLFQWSKWKKRMSMSSISSGSARRPVFDDKEDVNFDHFQILRAIGKGSFG
 KVCIVQKRDT EKMYAMKYMN KQQCIERDEV RNVFRELEIL QEIEHVFLVN LWY
 SFQDEED MFMVVDLLLG GDLRYHLQQN VQFSEDTVRL YICEMALALD YLRSQH
 I IHRDVKPDNILLDEQGH AHLTDF NIATIIKDGERATALAGTKP YMAPEIFHS
 FVNGGTGYSFEVDWWSVGVMAYELLRGWRPYDIHSSNAVESLVQLFSTVSVQY
 VPTWSKEMVALLRKLLTVNPEHRFSSLQDMQTAPSLAHVLWDDLSEKKVEPGF
 VPKGRRLHCDPTFELEEMILESRLPLHKKKKRLAKN KSRDSSRDSSQSENDYLQ
 DCLDAIQQDFVIFNREKLKRSQELMSEPPPGPETS DMTDSTADSEAEPTALPM
 CGSICPSSGSS

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Fig. 4

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ggcacgaggg  gcctatgagc  tgctgcgagg  atggaggccc  tatgacatcc  actccagcaa  60
cgccgtggag  tccctggtgc  agctgttcag  caccgtgagc  gtccagtatg  tccccacgtg  120
gtccaaggag  atggtggcct  tgctgcgga  gctcctcact  gtgaaccccg  agcacggct  180
ctccagcctc  caggacgtgc  aggcagcccc  ggcgctggcc  ggcgtgctgt  gggaccacct  240
gagcgagaa  aggttggagc  cgggcttcgt  gcccaacaaa  ggcgtctgc  actgcgaccc  300
cacctttgag  ctggaggaga  tgatcctgga  gtccaggccc  ctgcacaaga  agaagaagcg  360
cctggccaag  aacaagtccc  gggacaacag  cagggacagc  tccagtcgg  agaatgacta  420
tcttcaagac  tgcctcgatg  ccatccagca  agacttcgtg  attttaaca  gagaaaagct  480
gaagaggagc  caggacctcc  cgagggagcc  tctccccgcc  cctg  524

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Fig. 5

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tgcagttctc  cgaggacacg  gtgagggtgt  acatctgcga  gatggcactg  gctctggact  60
acctgcgcgg  ccagcacatc  atccacagag  atgtcaagcc  tgacaacatt  ctccctggatg  120
agagaggaca  tgcacacctg  accgacttca  acattgccac  catcatcaag  gacggggagc  180
ggcgacggc  attagcaggc  accaagccgt  acatggctcc  ggagatcttc  cactcttttg  240
tcaacggcgg  gaccggctac  tccttcgagg  tggactggtg  gtcggtgggg  gtgatggcct  300
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Fig. 6

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tcagattctc  cgaggacacg  gtgagggtgt  acatctgcga  gatggcactg  gctctggact  60
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gagaggacat  gcacacctga  ccgacttcaa  cattgccacc  atcatcaagg  acggggagcg  180
ggcgacggca  ttagcaggca  ccaagccgtg  acatggctcc  ggagatcttc  cactcttttg  240
tcaacggcgg  gaccggctac  tccttcgagg  tggactggtg  gtcggtgggg  gtgatggcct  300
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ctgggtgcag  ctgtttcagc  accgtgagcg  tccagtatg  tccccacgt  ggttccaagg  420
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ccagcttcca  ggacgttgca  ggcan  505

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-4/12 -

Fig. 7

tttttttttt	tttttctgtt	aaaaatcacg	aagtcttgct	ggatggcatc	caggcagtct	60
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gccagccgct	ttttctctct	gtgcagaggc	ctcgactcaa	ggatcatctc	ctccagctca	180
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ttgggaacagg	tgggcacgta	ctggacactc	acagtgtgta	acagctgaac	cagcgactcc	420
acagcattgc	tcgagtggat	atcgtaaggt	ctctgtgggc	agggaaagca		469

Fig. 8

MGGNHSHKPP	VFDENEEVNF	DHFQILRAIG	KGSFGKVCIV	QKRDTKKMYA	MKYMNKQKCI	60
ERDEVNRNVFR	ELQIMQGLEH	PFLVNLWYSF	QDEEDMFMVV	DLLLGDLRY	HLQONVHFTE	120
GTVKLYICEL	ALALEYLQRY	HIIHRDIKPD	NILLDEHGHV	HITDFNIATV	VKGAERASSM	180
AGTKPYMAPE	VFQVYMDRGP	GYSYPVDWWS	LGITAYELLR	GWRPYEIHVS	TPIDEILNMF	240
KVERVHYSST	WCKGMVALLR	KLLTKDPESR	VSSLHDIQSV	PYLADMNWD A	VFKKALMPGF	300
VPNKGRLNCD	PTFELEEMIL	ESKPLHKKKK	RLAKNRSRDG	TKDSCPLNGH	LQHCLETVRE	360
EFIIFNREKL	RRQQGQGSQ L	LDTDSRGGGQ	AQSKLQDGCN	NNLLTHTCTR	GCSS	414

Fig. 9

BLASTP - alignment of 281_gw1prot against tremblnew|AB041542|AB041542_1
 unnamed ORF; Mus musculus brain cDNA, clone MNCb-1563, similar to AJ250840
 serine/threonine protein kinase (Mus musculus). //:trernbl|AB041542|AB041542_1
 unnamed ORF; Mus musculus brain cDNA, clone MNCb-1563, similar to AJ250840
 serine/threonine protein kinase (Mus musculus). //:gp|AB041542|7670350 unnamed ORF;
 Mus musculus brain cDNA, clone MNCb-1563, similar to AJ250840 serine/threonine
 protein kinase (Mus musculus).

This hit is scoring at : 0.0 (expectation value)

Alignment length (overlap) : 365

Identities : 92 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Database searched : nrdb

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Q:      1  QVCIVQKRDTEKMYAMKYMNKQQCIERDEVNFFRELEILQEIEHVFLVNLWYSFQDEED
H:      107 KVCIVQKRDTEKMYAMKYMNKQQCIERDEVNFFRELEILQEIEHVFLVNLWYSFQDEED

          MFMVVDLLLGDDLRYHLQQNVQFSEDTVRLYICEMALALDYLRGQHI IHRDVKPDNILLD
          MFMVVDLLLGDDLRYHLQQNVQFSEDTVRLYICEMALALDYLR. QHI IHRDVKPDNILLD
          MFMVVDLLLGDDLRYHLQQNVQFSEDTVRLYICEMALALDYLRSQHI IHRDVKPDNILLD

          ERGHAHLTDFNIATIIKDGERTALAGTKPYMAPEIFHSFVNGGTGYSFEVDWWSVGVMA
          E:GHAHLTDFNIATIIKDGERTALAGTKPYMAPEIFHSFVNGGTGYSFEVDWWSVGVMA
          EQGHAHLTDFNIATIIKDGERTALAGTKPYMAPEIFHSFVNGGTGYSFEVDWWSVGVMA

          YELLRGWRPYDIHSSNAVESLVQLFSTVSVQYVPTWSKEMVALLRKLLTVNPEHRLSSLQ
          YELLRGWRPYDIHSSNAVESLVQLFSTVSVQYVPTWSKEMVALLRKLLTVNPEHR.SSLQ
          YELLRGWRPYDIHSSNAVESLVQLFSTVSVQYVPTWSKEMVALLRKLLTVNPEHRFSSLQ

          DVQAAPALAGVLWDHLSEKRVPEPGFVPNKGRLHCDPTFELEEMILESRPLHKKKKRRLAKN
          D:Q.AP:LA VLWD.LSEK:VEPGFVPNKGRLHCDPTFELEEMILESRPLHKKKKRRLAKN
          DMQTAPSLAHVLWDDLSEKKVEPGFVPNKGRLHCDPTFELEEMILESRPLHKKKKRRLAKN
  
```

Fig. 9 (continued)

KSRDNRDSSQSENDYLQDCCLDAIQQDFVIFNREKMKRSQDLPREPLPAPESRDAAEPVE
 KSRD:SRDSSQSENDYLQDCCLDAIQQDFVIFNREK:KRSQ:L .EP P.PE::D...:
 KSRDSSRDSSQSENDYLQDCCLDAIQQDFVIFNREKLRKSQELMSEPPPGPETSMTDSTA

D-EAE	364
D EAE	
DSEAE	471

Two conserved signatures for eukaryotic protein kinases are indicated in bold. Aspartic acid (D) of the active site is shown underlined within the bold area of the Protein Kinase ST pattern. The ATP binding site (K) is shown underlined within the bold ATP binding region. The 5' portion of the sequence is missing in this incomplete sequence .

PROTEIN KINASE ST region, residue 107 to 120 [prosite].
 Eukaryotic protein kinase domain region, residue 1 to 240 [pfamsearch].

Fig. 10

BLASTP - alignment of 281_gw1prot against trembl|AJ250839|HSA250839_1

product: "serine/threonine protein kinase"; Homo sapiens gene for serine/ threonine protein kinase //:gp|AJ250839|7160989 product: "serine/threonine protein kinase"; Homo sapiens gene for serine/threonine protein kinase.

This hit is scoring at : 5e-145 (expectation value)
 Alignment length (overlap) : 340
 Identities : 69 %
 Scoring matrix : BLOSUM62 (used to infer consensus pattern)
 Database searched : nrdb

Q: 1 QVCIVQKRDTKMYAMKYMKNKQCIERDEVNRFREILQIEHVFVNLWYSFQDEED
 :VCIVQKRDT:KMYAMKYMKNKQ:CIERDEVNRFREL:I:Q:EH FLVNLWYSFQDEED
 H: 36 KVCIVQKRDTKMYAMKYMKNKQCIERDEVNRFRELQIMQGLEHVPFLVNLWYSFQDEED
 MFMVVDLLGGDLRYHLQQNVQFSEDTVRLYICEMALALDYLRGQHI IHRDVKPDNILLD
 MFMVVDLLGGDLRYHLQQNV.F:E.TV:LYICE:ALAL:YL: .HI IHRD:KPDNILLD
 MFMVVDLLGGDLRYHLQQNVHFTGTVKLYICELALALEYLQRYHI IHRDIKPDNILLD
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 E.GH.H:TDFNIAT::K..ERA:::AGTKPYMAPE:F::: G.GYS::VDWWS:G:.A
 EHGHHITDFNIATVVVKGAEERASSMAGTKPYMAPEVFQVYMDRGPYSYPVDWWSLGITA
 YELLRGWRPYDIHSSNAVESLVQLFSTVSQYVPTWSKEMVALLRKLITVNPEHRLSSLQ
 YELLRGWRPY:IHSF.. .V.Y .TW.K MVALLRKLIT :PE.R:SSL.
 YELLRGWRPYEIHSTVPIDEILNMFKVERVHYSSSTWCKGMVALLRKLITKDPESRVSSLH
 DVQAAPALAGVLWDHLSEKRVPEPGFVPNKGRLHCDPTFELEEMILESRLHKKKKRLAKN
 D:Q:.P LA.: WD : :K.: PGFVPNKGRL:CDPTFELEEMILES:PLHKKKKRLAKN
 DIQSVPYLADMNWDVAFKKALMPGFVPNKGRLNCDPTFELEEMILESPLHKKKKRLAKN
 KSRDNSRDSSQSENDYLQDCLDAIQQDFVIFNREKMKRSQ 340
 :SRD.:DS. N.:LQ.CL:.:F:IFNREK:R.Q
 RSRDGTKDSCPL-NGHLQHCLFTVREEFIIFNREKLRRQQ 374

Fig. 11

HMMPFAM - alignment of 281_gwlprot against pfam|hmm|pkinase

Eukaryotic protein kinase domain

This hit is scoring at : 205.7

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

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      :V  :::: T K::A:K::K:::
      :RE::IL:::H :V.L F:::
      :lrEiqilkrIsHpNIvrllgvfedtddd

DMFMVVDLLLGDLRYHLQQNVQFSEDTVRLYICEMALALDYLRGQHI IHRDVKPDNILL
:::V::: GGD L :L:N ..SE...: ::::: .L:YL... I:HRD:KP:NILL
hlylvmEymegGdLfdyllrrngplsekeakialQilrGleyLHsngivHRDLKpeNILL

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DE.G ::.DF::A::: E:T...GT. YM APE::: .G .GYS :VD WS:GV
dengtVKiaDFGLArll...eklttfvGTpwYmmAPEvi...leg.rgysskvDvWSlGv

MAYELLRG-----WRPYD-----IHSSNAVESLVQLf
:YELL.G
iLyEl1tgglfpgadlpaftggdevdqliifvlklPfsdelpktridpleelfrikkr.

stvSVQYVPTWSKEMVALLRKLLTVNPEHRL---SSLQ      240
:::.. S:E: LL:K.L. :P.:R :::
...rlplpsncSeelkdLLkkcLnkDpskRpGsatake      271
```

Fig. 12

BLASTP - alignment of 281_gwlprot against pdb|1CDK|1CDK-A
cAMP-dependent protein kinase(protein kinase a)protein kinase inhibitor(pki(5-24))

This hit is scoring at : 4e-39 (expectation value)
Alignment length (overlap) : 274
Identities : 32 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

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H: 49 RVMLVKHKETGNHFAMKILDKQKVVKLKQIEHTLNEKRILQAVNFPFLVKLEYSFKDNSN

   MFMVVDLLLGGDLRYHLQONVQFSEDTVRLYICEMALALDYLRGQHI IHRDVKPDNILLD
   :MV.:.:GG.:HL.:FSE..R.Y.:.:L.:YL.:.:I:RD:KP:N:L:D
   LYVMVEYVPGGEMFSLRRIGRFSEPHARFYAAQIVLTFEYLSLDLIYRDLKPENLLID

   ERGHAHLTDFNIATIIKDGERTALAGTKPYMAPEIFHSFVNGGTGYSFEVDWWSVGVMA
   :G.:.:TDF..A.:K. R..L.GT..Y:APEI. S .GY: .VDWW:GV:
   QQGYIQVTDGFGFAKRVKG--RTWTLCGTPEYLAPEIILS-----KGYNKAVDWMALGVLI

   YELLRGWRPYDIHSSNAVESLVQLFSTV--SVQYVPTWSKEMVALLRKLLTVNPEHRLS
   YE:..G: P: .:..:Q:.:.:.:V.:.:S:..:LLR.LL.V:..R.:
   YEMAAGYPPF-----FADQPIQIYEKIVSGKVRFPSPHFSSDLKDLLRNLLQVDLTKRFG

   SLQ-----DVQAAPALAGVLWDHLSEKRVPEPGFVP      267
   :L: D:..: .A. W : :.:VE. F:P
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Fig. 13

Expression of human serine-threonine kinase in tissues relevant for cancer

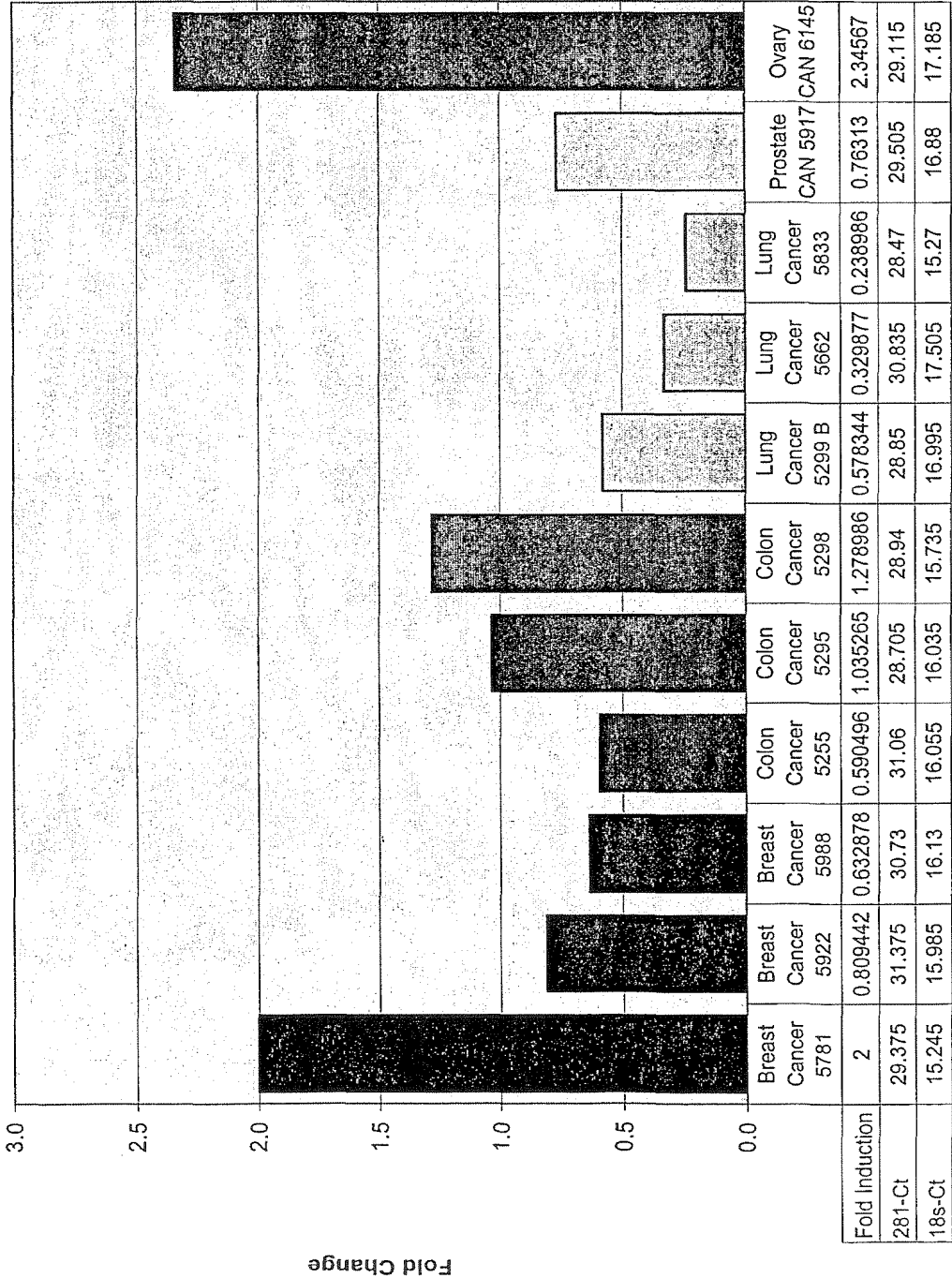


Fig. 14a Expression of human serine-threonine protein kinase in tissues relevant for obesity and diabetes

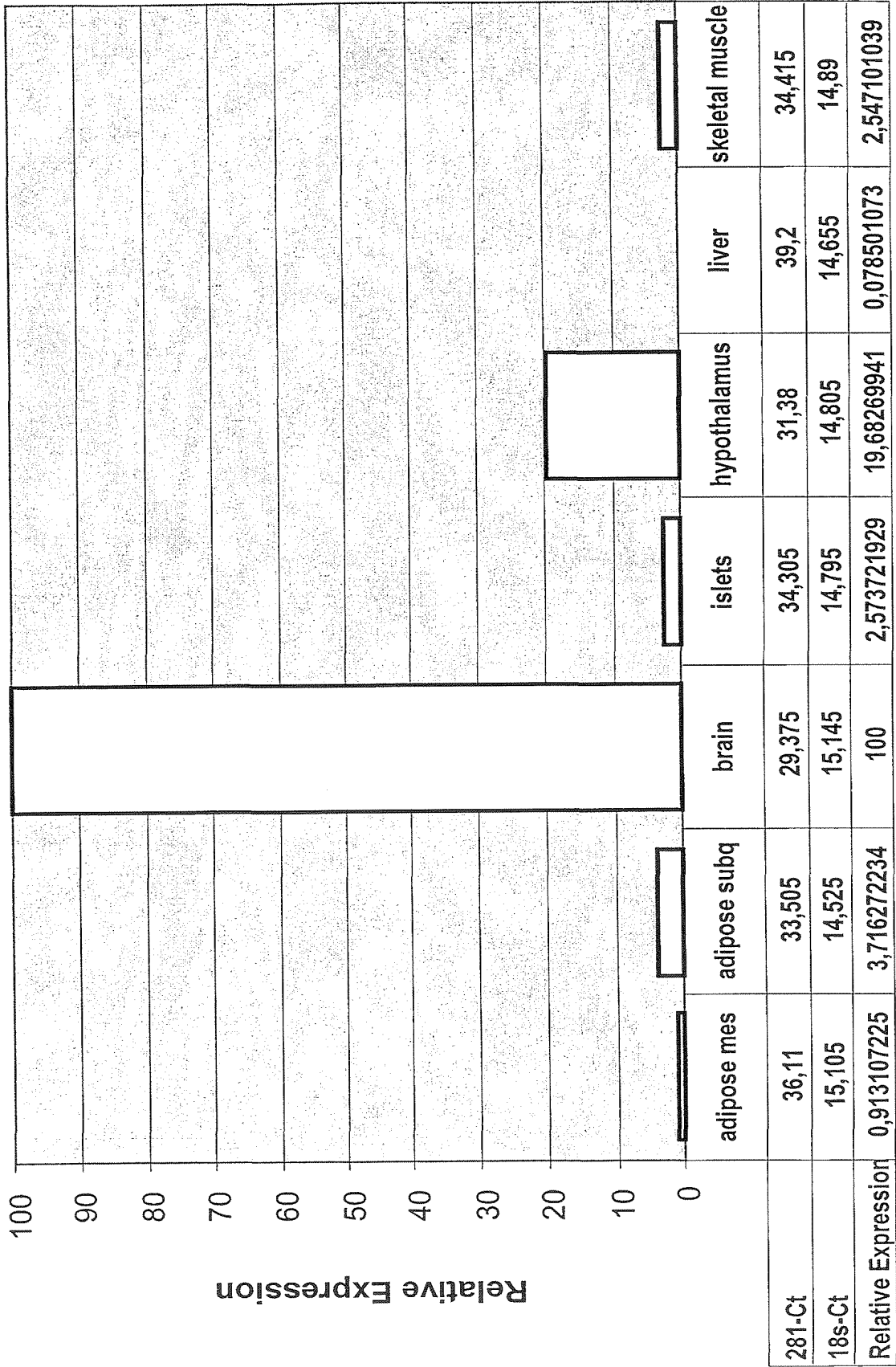
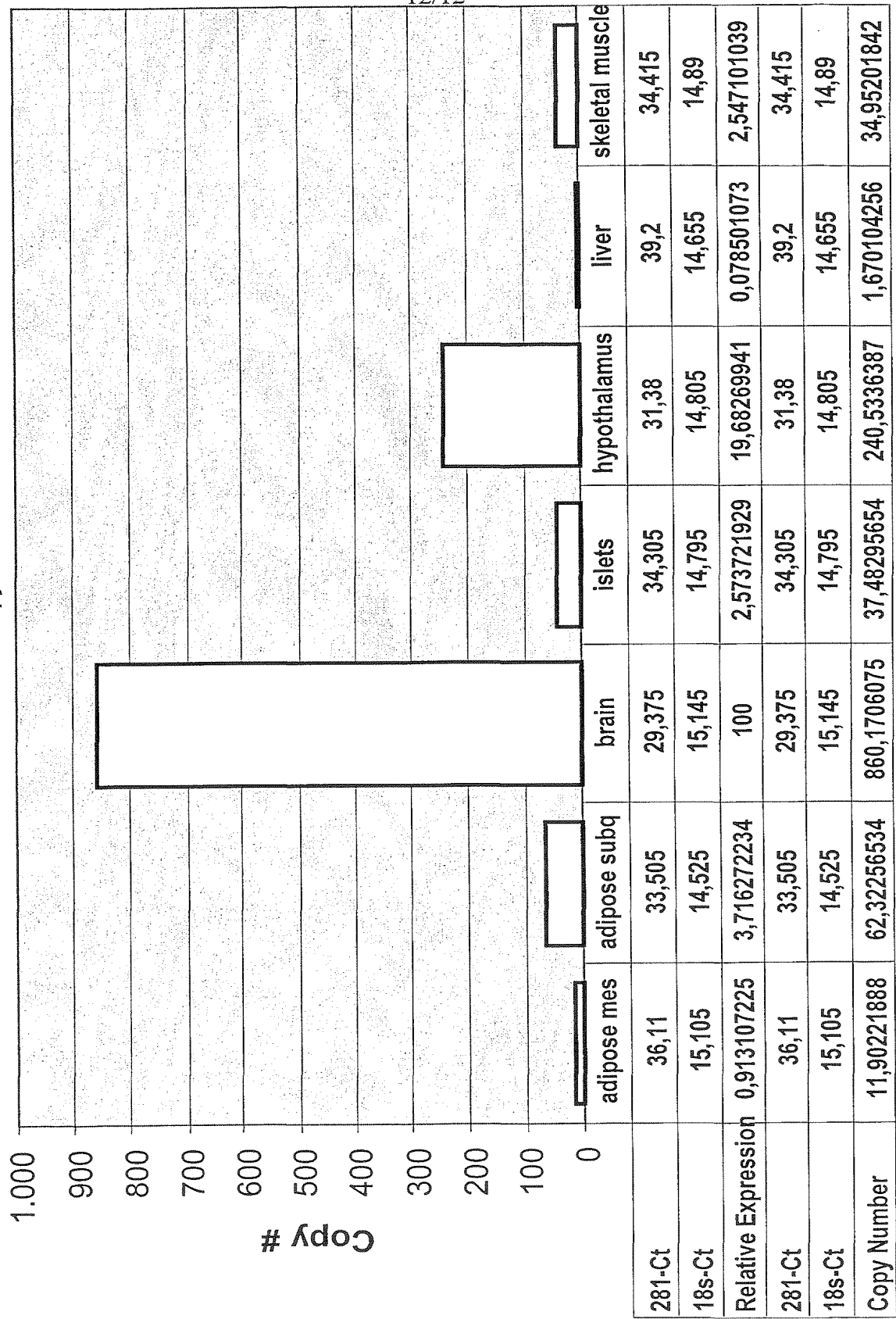


Fig 14b Expression of human serine-threonine protein kinase in tissues relevant for obesity and diabetes



- 1 -

SEQUENCE LISTING

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<130> LIO185 Foreign Countries

<150> US 60/240,097

<151> 2000-10-16

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Ser	Glu	Lys	Arg	Val	Glu	Pro	Gly	Phe	Val	Pro	Asn	Lys	Gly	Arg	Leu	
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His	Cys	Asp	Pro	Thr	Phe	Glu	Leu	Glu	Glu	Met	Ile	Leu	Glu	Ser	Arg	
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Leu Asp Ala Ile Gln Gln Asp Phe Val Ile Phe Asn Arg Glu Lys Met
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Lys Lys Arg Met Ser Met Ser Ser Ile Ser Ser Gly Ser Ala Arg Arg
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Pro Val Phe Asp Asp Lys Glu Asp Val Asn Phe Asp His Phe Gln Ile
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Thr Val Arg Leu Tyr Ile Cys Glu Met Ala Leu Ala Leu Asp Tyr Leu
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Arg Ser Gln His Ile Ile His Arg Asp Val Lys Pro Asp Asn Ile Leu
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Leu Asp Glu Gln Gly His Ala His Leu Thr Asp Phe Asn Ile Ala Thr
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Ile Ile Lys Asp Gly Glu Arg Ala Thr Ala Leu Ala Gly Thr Lys Pro
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260 265 270

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atgagctgct gcgaggatgg aggccctatg acatccactc cagcaacgcc gtgggagtcc	360
ctgggtgcag ctgtttcagc accgtgagcg ttccagtatg ttccccacgt ggttccaagg	420
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<211> 469

<212> DNA

<213> Homo sapiens

<400> 7

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gccagccgct tttttcttctt gtgcagagggc ctcgactcaa ggatcatctc ctccagctca      180
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tcactcaggt cgtccacag cacgtggggc agcgatgggg ctgtctgcat gtcttggaga      300
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Met Gly Gly Asn His Ser His Lys Pro Pro Val Phe Asp Glu Asn Glu
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Glu Val Asn Phe Asp His Phe Gln Ile Leu Arg Ala Ile Gly Lys Gly
          20          25          30

```

```

Ser Phe Gly Lys Val Cys Ile Val Gln Lys Arg Asp Thr Lys Lys Met
          35          40          45

```

```

Tyr Ala Met Lys Tyr Met Asn Lys Gln Lys Cys Ile Glu Arg Asp Glu
50          55          60

```

```

Val Arg Asn Val Phe Arg Glu Leu Gln Ile Met Gln Gly Leu Glu His
65          70          75          80

```

```

Pro Phe Leu Val Asn Leu Trp Tyr Ser Phe Gln Asp Glu Glu Asp Met
          85          90          95

```

```

Phe Met Val Val Asp Leu Leu Leu Gly Gly Asp Leu Arg Tyr His Leu
          100          105          110

```

```

Gln Gln Asn Val His Phe Thr Glu Gly Thr Val Lys Leu Tyr Ile Cys
          115          120          125

```

- 10 -

Glu Leu Ala Leu Ala Leu Glu Tyr Leu Gln Arg Tyr His Ile Ile His
 130 135 140

Arg Asp Ile Lys Pro Asp Asn Ile Leu Leu Asp Glu His Gly His Val
 145 150 155 160

His Ile Thr Asp Phe Asn Ile Ala Thr Val Val Lys Gly Ala Glu Arg
 165 170 175

Ala Ser Ser Met Ala Gly Thr Lys Pro Tyr Met Ala Pro Glu Val Phe
 180 185 190

Gln Val Tyr Met Asp Arg Gly Pro Gly Tyr Ser Tyr Pro Val Asp Trp
 195 200 205

Trp Ser Leu Gly Ile Thr Ala Tyr Glu Leu Leu Arg Gly Trp Arg Pro
 210 215 220

Tyr Glu Ile His Ser Val Thr Pro Ile Asp Glu Ile Leu Asn Met Phe
 225 230 235 240

Lys Val Glu Arg Val His Tyr Ser Ser Thr Trp Cys Lys Gly Met Val
 245 250 255

Ala Leu Leu Arg Lys Leu Leu Thr Lys Asp Pro Glu Ser Arg Val Ser
 260 265 270

Ser Leu His Asp Ile Gln Ser Val Pro Tyr Leu Ala Asp Met Asn Trp
 275 280 285

Asp Ala Val Phe Lys Lys Ala Leu Met Pro Gly Phe Val Pro Asn Lys
 290 295 300

Gly Arg Leu Asn Cys Asp Pro Thr Phe Glu Leu Glu Glu Met Ile Leu
 305 310 315 320

Glu Ser Lys Pro Leu His Lys Lys Lys Lys Arg Leu Ala Lys Asn Arg
 325 330 335

Ser Arg Asp Gly Thr Lys Asp Ser Cys Pro Leu Asn Gly His Leu Gln
 340 345 350

His Cys Leu Glu Thr Val Arg Glu Glu Phe Ile Ile Phe Asn Arg Glu
 355 360 365

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Lys Leu Arg Arg Gln Gln Gly Gln Gly Ser Gln Leu Leu Asp Thr Asp
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Ser Arg Gly Gly Gly Gln Ala Gln Ser Lys Leu Gln Asp Gly Cys Asn
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Asn Asn Leu Leu Thr His Thr Cys Thr Arg Gly Cys Ser Ser
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22

